



Nesting biology of an Oriental carpenter bee, *Xylocopa (Biluna) nasalis* Westwood, 1838, in Thailand (Hymenoptera, Apidae, Xylocopinae)

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Abstract

The biological study of wild non-*Apis* bees can provide useful information that may help with the pollination of food crops and native plants in areas where the keeping of honey bee colonies is restricted or affected by CCD. Here, we describe the nesting biology of the Oriental large carpenter bee, *Xylocopa (Biluna) nasalis* Westwood, 1838. An aggregation of more than 80+ bamboo nests of *X. nasalis* was discovered in Suan Pheung district, Ratch Buri province, Thailand on the 25th of May 2012. We collected 27 nests from the site to dissect, measure the external and internal nest architecture, and analyze the pollen composition of the pollen masses. *X. nasalis* constructs linear unbranched nests with nest entrance mostly located at the open-end of the bamboo culms. The nest length and the branch diameter of the nest entrance (excluding nesting edge) are 25.40 ± 6.95 cm and 17.94 ± 6.00 mm, and the maximum number of provisioned cells is 8. A biased sex ratio of 8♀: 1♂ is reported, with up to 7 adults inhabiting in a single nest. 29 pollen types were identified from 14 pollen masses using an acetolysis method and visualization under both light microscope and scanning electron microscope. 13 pollen types were considered as major pollen sources (contribute $\geq 1\%$ in total pollen volume); however, only 10 can be identified to family and generic levels. The dominant pollen sources are of the families Elaeagnaceae (*Elaeagnus* cf. *latifolia*), Euphorbiaceae (*Croton*), Fabaceae (*Senna siamea* and *Cassia*), Fagaceae (*Lithocarpus* and *Castanopsis*), and Lythraceae (*Trapa*) which are mostly native to the region of Southeast Asia. The nesting architectural details should prove to be beneficial to beekeepers and researchers who are interested in trapping and studying *X. nasalis*, and the polylectic behavior of *X. nasalis* can be highly valuable for future crop pollination strategies, particularly for plants that require sonication of their poricidal anthers.

Keywords

Carpenter bee, nesting biology, Thailand, bamboo, pollen

Introduction

Because of the declining honey bee population worldwide resulting from the condition known as Colony Collapse Disorder (CCD; Oldroyd 2007, van Engeldorf et al. 2008, Ratnieks and Carreck 2010), the use of widespread pesticides (Hopwood et al. 2012), climate changes (Bartomeus et al. 2011), and the increase in monotonous agricultural landscapes that reduce the biodiversity and the availability of foods for bees, the study of wild and/or domesticated non-*Apis* bees can provide useful information for complementary bee species that may help with the pollination of food crops in areas where keeping of honey bees colonies are being affected or restricted (Chagnon et al. 1993, Wilmer et al. 1994, Javorek et al. 2002, Hoehn et al. 2008, Brittain et al. 2013). Until now, only a handful of non-*Apis* bee species have been used extensively in agriculture, e.g., *Bombus terrestris* (Linnaeus, 1758), *Megachile rotundata* (Fabricius, 1787), *Nomia melanderi* Cockerell, 1906, *Osmia rufa* (Linnaeus, 1758), and some stingless bee species (Westerkamp and Gottsberger 2000, Hogendoorn et al. 2006, Greenleaf and Kremen 2006, Slaa et al. 2006, Hoehn et al. 2008). These bees have been shown to be effective pollinators, as good as, if not better than, honey bees on certain crop plants (Greenleaf and Kremen 2006).

The large carpenter bees of the genus *Xylocopa* Latreille, 1802 (Hymenoptera; Apoidea) have recently received attention due to their pollination capabilities. The use of large carpenter bees to assist with pollination of greenhouse tomatoes and honeydew melons in Australia and Israel has been reported (Hogendoorn et al. 2000, Sadeh et al. 2007, Keasar 2010). In Brazil, where passion fruits are one of the main exported fruit crops of the nation, studies of using *X. (Neoxylocopa) grisescens* Lepeletier, 1841 and *X. (N.) frontalis* (Olivier, 1789) to pollinate the flowers, instead of using manual labor, have shown promising results in increasing the fruit sets and quality and reducing the production costs (Junqueira et al. 2012, Yamamoto et al. 2012).

Carpenter bees can be found throughout the tropical and subtropical parts of the world (Hurd and Moure 1963, Gerling et al. 1989). These are large and robust bees that many novices regularly confuse with bumble bees (*Bombus*) due to their similar sizes and shapes. There are currently ca. 470 species described with 32 subgenera recognized in a single genus (Michener 2007, Ascher and Pickering 2013). Most *Xylocopa* species are known to excavate their nests in dead or decaying woods, with the exception of the subgenus *Proxylocopa* Hedicke, 1938 which excavates nests in the soil (Gottlieb et al. 2005). There are two main types of nests among the wood-nesting *Xylocopa* species: (1) unbranched or linear nests in which the tunnel runs in the same direction as the nest entrance or at most with a single right angle corner from the nest entrance

and (2) branched nests which consist of at least two tunnels or more although with only one nest entrance (Gerling et al. 1989).

One subgenus of Oriental *Xylocopa*, *Biluna* Maa, 1938, comprises five to nine species (Michener 2007, Ascher and Pickering 2013). Its distribution ranges from India and Sri Lanka to Southeast Asia and Japan. Species of *Biluna* are only known to construct unbranched nest in bamboo culms (Maa 1946, Maeta et al. 1985; Hurd and Moure 1963). *Xylocopa (Biluna) nasalis* Westwood, 1838, is a species commonly found throughout Southeast Asia. It superficially resembles the sympatric species *X. (Mesotrichia) latipes* Drury, 1773 and *X. (M.) tenuiscapa* Westwood, 1840 because of the presence of black pubescence on the mesosoma and their large size (21–35 cm in length). Males of *Biluna* lacks both a basitibial plate and a spine on the outer apex of the hind tibia, while the females have a dense mat of short setae on the middle tibia and lack an apical middle tibial spine. The behavior, biology and natural history of *X. nasalis*, is poorly known even though it is commonly found throughout rural and agricultural areas in Southeast Asia. Boontop et al. (2008) briefly described the nesting biology of *X. nasalis* studied in Kasetsart University, Kamphaengsaen Campus, Nakhonpathom province, Thailand, though their account lacks many nest architectural details and, importantly, the palynological data on plant food sources. Here, we extend Boontop et al. (2008)'s work via reporting the finding of a nest aggregation of *X. nasalis* in Suan Pheung district, Ratch Buri province (~100 km southeast of Nakhonpathom province), Thailand, along with details of other nest architectural components, its floral preferences, and some behavioral observations at the nest entrance. We anticipate that by providing such detailed nesting biology and pollen food sources of a local large carpenter bee from an area with poorly known mellitological data (such as Southeast Asia), it will stimulate interest and provide practical information for local bee keepers and bee researchers to consider *Xylocopa* to be an important native pollinator for certain crops and endemic plants in the near future.

Methods

Nesting site and nest dissections

We discovered a nesting aggregation of *Xylocopa nasalis* in a makeshift roof structure (Figure 1; 80+ nests) made from bamboo culms (tribe Bambuseae) at a local restaurant in Suan Pheung district, Ratch Buri province, Thailand (13°33'32.4138"N and 99°21'32.3202"E) on the 25th of May 2012. The collecting of the nests was done in the early month of the Monsoon season in Thailand, when flowers were abundantly blooming. Almost 95% of the bamboo culms were occupied by *X. nasalis*. We also observed some unidentified megachilids using some of the bamboo culms for nesting as well. The bamboo culms ranged in size of outer diameter ≈ from 15.80 to 29.39 mm and 3.00 to 3.25 m in length (n = 27) and were situated approximately 2.50 m above the ground. We collected 27 nests (with all of the bee inhabitants) by plugging the nest



Figure 1. Nesting habitat of *X. nasalis*; A nesting habitat of *X. nasalis* on a makeshift roof of a restaurant in Suan Pheung district, Ratch Buri province, Thailand. The red arrows indicate locations where the bamboo culms were arranged ca. 2.50 m above the ground (**1a** and **1b**). At the nest entrance, the female of *X. nasalis* was dehydrating the nectar previously foraged (**1c**).



Figure 2. Nesting architecture of *X. nasalis*; Dissected nests of *X. nasalis* revealing the nest structure inside the bamboo culm and its residents. Measurements of the nest parameters are shown in Table 1. The diameters of the nests (excluding the nest thickness) were measured at the nest entrance, followed by the vestibulum (antechamber) length, cell length, and the inner most cell length, respectively (**2a**). Cells containing larvae with pollen masses and their feces were collected and weighted (**2b**).

entrances with cotton balls and sealing them with duct tape. Nests were brought back to the Department of Biology, Chulalongkorn University, Thailand, and preserved in a -20 °C freezer for later dissection. We dissected each nest and recorded the following

data: measurements of the external and internal nest structures using a vernier caliper and tape measurement (Figure 2), numbers of individuals at different life-stages, numbers of provisioned cells, fresh weights of the pollen balls in each cell, feces' weights, and sex ratio of adults. Since the data on *X. nasalis* cell lengths cannot be assumed to be drawn from any given probability distribution, the non-parametric Kruskal-Wallis test was employed to test whether there are differences among the average cell lengths of 54 cells measured; the Mann-Whitney U test was used to test the difference between the average inner most cell length of all 27 nests and the average cell lengths. The two statistical tests were performed using the program SPSS ver. 20.0 (IBM Corp. 2001). Fourteen pollen balls from six nests were collected for pollen analysis. All remaining nests were sketched and photographed. All specimens (including eggs, larvae, and pupae) were preserved in 95% Ethanol and deposited at the Natural History Museum of Chulalongkorn University, Bangkok, Thailand for future genetic analyses.

Pollen analyses

For pollen analysis, we employed the acetolysis method (Erdtman 1960) with a modification at the end of the process where we re-suspended the decanted pollen samples in a benzene solution and topped up with silicone oil in a vial. The pollen-benzene solution was air-dried for one week or until the benzene solution was completely evaporated leaving only the pollen samples preserved in silicone oil for examination under light microscope (Olympus CH-BI45-2). For preparing the pollen pictures to be captured and identified under a scanning electron microscope (Hitachi Tabletop Microscope TM-100), an additional step was performed before pollen samples were re-suspended in benzene solution: 70% ethanol was added and the pollens were mixed in the solution, the pollen was pelleted by centrifugation, and the ethanol was then discarded (we sometimes repeated additional steps with 95% and absolute ethanol).

Before counting the pollen grains, we mixed the vial containing pollen grains submerged in silicone oil to obtain a homogenous pollen suspension. Ten drops of the pollen suspension were removed and placed on microscopic slides and each aliquot was spread to an area of ca. 30×30 mm. Three slides per pollen mass were used for the examination. We counted 300 pollen grains for each slide, which provide a total pollen count of 900 grains from a single pollen mass. Since there is no published exhaustive key for the pollens endemic to western Thailand, we were limited in resources to accurately identify most pollens to the specific level. We followed the pollen identification guides from various authors whose works were on the melittopalynology of the Asian Tropics, i.e., Huang (1972), Tissot et al. (1994), Nagamitsu et al. (1999), and Jongjitvimon and Wattanachaiyingcharoen (2006), which allowed us to identify most pollen types to family and genus. We also identified the plant food sources in the area of western Thailand using published botanical keys provided by Hanum and van der Maesen (1977), Gardner et al. (2000), Smitinand (2001), and Phengklai (2006), to

corroborate with our pollen data. The plant classification system of the Angiosperm Phylogeny Group (2009) was followed.

Since pollens are diverse in their shapes and sizes, to accurately identify which pollen type contributes the most to the bee diets, one should not depend only on the most number of grain counts alone. Buchmann and O'Rourke (1991) suggested weighing the volume of pollens with the percentage of the pollen counts to achieve a more reliable estimation of the type of pollens that contribute to the pollen masses. To obtain the volumes of each pollen type, we measured the longitudinal axis (p) and equatorial axis (e) lengths from 30 grains of each pollen type then calculated the mean values. Pollen dimensions are categorized into two types: spherical and elliptical forms. The following formulas were used for the calculation of the pollen volumes: spherical form = $1/6\pi p^3$ and elliptical form = $1/6\pi e^2 p$. Contributing pollen types were subjectively categorized into two groups – the “major” and “minor” pollen sources based on their percentage of total volume. The major pollen sources are defined as contributing in *X. nasalis* diet $\geq 1\%$ of the total pollen volume, whereas minor pollen sources are those that are accounted $< 1\%$ of the total pollen volume.

We also observed some behaviors exhibited by the bees on the day before we collected the bamboo nests. These behaviors were related to their nesting habits and are briefly discussed in the next section.

Results

Nest architecture and contents

Nests of *Xylocopa nasalis* nest are strictly unbranched. The provisioned cells are separated by partitions made from bamboo particles excavated by the founding female. All of the nest entrances are located at the end of the bamboo culms, except for a couple of nests that the bees excavated from the undersides. A summary of nest architectural details is provided in Table 1. The average total nest length (including the vestibulum (antechamber) length) is 38.35 cm. The average nest length (measured from the nest entrance to the end of the innermost cell) is ca. 25.40 ± 6.95 cm. The mean branch diameter of the nests (excluding nest thickness) is 17.94 ± 6.00 mm. The number of cells per nest ranged from 0–8 cells with an average cell number around 3 per nest. There is a difference in terms of the average individual cell lengths among cells from 27 nests ($\chi^2 = 28.11$, $p = 0.021$), though the significance value is fairly weak. On the contrary, the innermost cell lengths were tested to be strongly different from other cells ($U < 0.0001$, $p < 0.0001$). The average number of individual adult bees found per nest ranged between 1 and 7 individuals (mean \pm s. d.: 3.24 ± 1.90) with a sex-ratio bias of $7.98\text{♀: }1\text{♂}$. We found the average number of pupae and post-defecating larvae: larvae: eggs as follow 1.15: 0.69: 0.04; however, we did not find any nest that had all life stages of the bees present at once. Three of the 27 nests contained eggs; the mean fresh weight of their

Table 1. Nesting structure measurements of *X. nasalis*; Summary of the measurements of nesting architecture of *X. nasalis* (n = 27) from Suan Pheung district, Ratch Buri province, Thailand (13°33' 32.4138"N and 99°21'32.3202"E).

Nest Characters	Ranges	Max.	Min.
	(Mean \pm S.D.)		
Vestibulum length (cm)	12.95 \pm 67.80	31.00	5.04
Nest length (cm)	25.40 \pm 6.95	36.25	10.00
Number of cells / nest	2.83 \pm 2.55	8	0
Inner most cell length* (mm)	32.75 \pm 11.06	55.30	15.00
Individual cell length (except *) (mm)	23.25 \pm 3.88	41.00	17.00
Branch diameter at nest entrance (mm)	17.94 \pm 6.00	30.70	11.00
Nest thickness (mm; measured at the entrance)	4.66 \pm 0.79	6.80	3.30
Partition thickness (mm)	0.88 \pm 0.27	1.60	0.50
Pollen weight / cell (g) (n = 3)	1.37 \pm 0.13	1.52	1.20
Feces' weight / cells (g) (n = 6)	0.24 \pm 0.23	0.86	0.01
Number of adult individuals	3.24 \pm 1.90	7	1
Number of female adults	3.19 \pm 2.04	7	1
Number of male adults	0.40 \pm 0.70	2	0
Number of pupa and post-defecating larva	1.15 \pm 2.41	7	0
Number of larva	0.69 \pm 1.38	5	0
Number of eggs	0.04 \pm 0.19	3	0

unconsumed pollen masses was 1.37 ± 0.13 g (n = 3). The average weight of the feces in the cells of post-defecating larvae averaged 0.24 ± 0.23 g (n = 6).

Pollen analyses

A total of 29 pollen types were identified from the 14 pollen masses. We were able to identify pollen grains from 13 families, including 12 identifiable genera (Table 2). For three of the 13 plant families – Anacardiaceae, Araceae, and Cyperaceae – generic level identification could not be confirmed. Brief descriptions of the 14 unidentified pollen types are also given in Table 2. We consider 13 pollen types as “major” pollen sources, whereas the other 16 pollen types are considered as “minor” pollen sources based on their percentage total volumes of the diets (Table 2, Figure 3).

For the 13 pollen types classified as major pollen sources, we were able to identify 10 pollen types to their generic level and 2 of these to species (*Elaeagnus* cf. *latifolia* Linnaeus and *Senna siamea* (Lam.) Irwin et Bradley). These include the family Acanthaceae (*Thunbergia*; 2.35%), Anacardiaceae (genus unknown; 4.68%), Elaeagnaceae (*E. cf. latifolia*; 12.88%), Euphorbiaceae (*Croton*; 14.95%), Fabaceae (*Cassia*; 12.17% and *S. siamea*; 12.91%), Fagaceae (*Lithocarpus*; 7.65% and *Castanopsis*; 3.22%), Lythraceae (*Trapa*; 13.36%), and Theaceae (*Schima*; 6.42%). Three pollen types (all are < 3% of total pollen volume) remain unidentified at any level (under “Unknowns” in

Table 2. Pollen type amount and volume foraged by *X. nasalis*; Percentage pollen grain count and percentage pollen volume frequently encountered from 14 pollen masses collected by *X. nasalis* from 6 nests. For each pollen mass, 900 pollens were counted (total of 12,600 pollen grains).

Family	Genus/Species	Grains counted	Approximate geometric figure of pollen	p	e	v	Percentage of pollen grains*	Total pollen volume by taxon** ($\times 10^{-7}$ cm 3)	Percentage of total pollen volume***
FAGACEAE									
<i>Lithocarpus</i>	3 310	Elliptic	17.13	23.02	4.71	26.43	155.57	7.65	
<i>Castanopsis</i>	1 724	Elliptic	15.05	22.01	3.8	13.77	65.51	3.22	
FABACEAE									
<i>Senna siamea</i>	1 045	Elliptic	30.09	40.11	25.12	8.35	262.5	12.91	
<i>Cassia</i>	751	Elliptic	32.53	44.09	32.93	6	247.3	12.17	
ELAEAGNACEAE									
<i>Elaeagnus</i> cf. <i>latifolia</i>	1 857	Elliptic	22.14	34.9	14.1	14.83	261.84	12.88	
LYTHRACEAE									
<i>Trapa</i>	655	Elliptic	33.04	49.1	41.47	5.23	271.63	13.36	
THEACEAE									
<i>Schima</i>	576	Elliptic	30.17	37.98	22.67	4.6	130.58	6.42	
ANACARDIACEAE	369	Elliptic	34.93	37.57	25.76	2.95	95.05	4.68	
EUPHORBIACEAE									
<i>Croton</i>	349	Sphere	55.01	NA	87.07	2.8	303.87	14.95	
JUNGLANDACEAE									
<i>Engelhardtia</i>	111	Elliptic	20.87	19.23	3.97	0.87	4.41	0.22	
ARACEAE	63	Elliptic	27.44	42.56	25.99	0.5	16.37	0.81	
RHAMNACEAE									
<i>Ziziphus</i>	60	Sphere	27.51	25.02	4.19	0.48	2.51	0.12	
ACANTHACEAE									
<i>Thunbergia</i>	48	Sphere	57.5	NA	99.5	0.38	47.76	2.35	
CAPRIFOLIACEAE									
<i>Sambucus</i>	37	Elliptic	19.98	30.03	9.42	0.3	3.49	0.17	
CYPERACEAE	13	Half sphere	25.00	NA	4.09	0.1	0.53	0.03	

Family	Grains counted	Approximate geometric figure of pollen	p	e	v	Percentage of pollen grains*	Total pollen volume by taxon** ($\times 10^{-7} \text{ cm}^3$)	Percentage of total pollen volume***
UNKNOWNNS								
Triangular, tripolate	595	Elliptic	27.44	25.08	8.99	4.75	53.49	2.63
Irregular shape, inaperture	368	Half sphere	20.1	NA	2.09	2.94	7.69	0.38
Monolete	226	Elliptic	24.98	42.57	23.63	1.8	53.4	2.63
Three furrows, triangular, tricoplate	185	Elliptic	20.03	22.51	4.71	1.48	8.71	0.43
Three furrows, tricoplate	180	Sphere	35.02	NA	22.44	1.44	40.39	1.99
Oblate, two pores fused, monolete								
Inaperture								
Triangular, inaperture								
Triporate								
Triangular, tripolate	78							
Fenestrated								
Inaperture								
Oblate, triangular, fenestrated								
Three bladders, vesiculate	12 600						100	2032.6
								100

p: mean longitudinal axis (μm); e: mean equatorial axis (μm); v: mean individual grain volume ($\times 10^{-9} \text{ cm}^3$)

* Percentage of pollen grains was calculated excluding the 78 unknowns pollen grains (thus the total number of grain for calculation was 12,522)

** Total pollen volume by taxon was calculated by multiplying the number of pollen grains by the mean individual grain volume

*** Percentage of total pollen volume was calculated excluding the 78 unknowns pollen grain volumes (thus the total pollen volume was $2032.60 \times 10^{-7} \text{ cm}^3$)

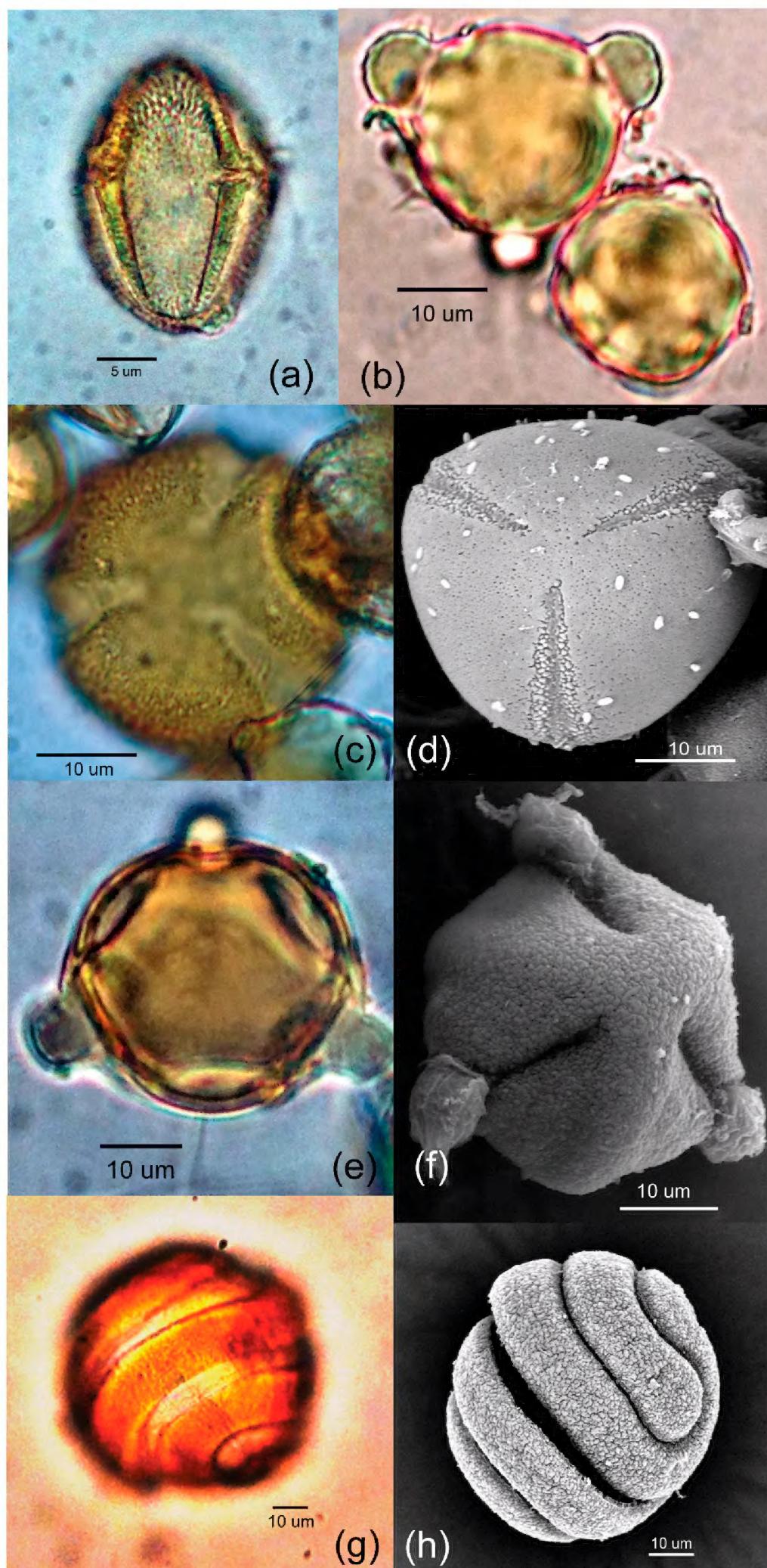


Figure 3. Pollen grains collected by *X. nasalis*; Some representations of pollens collected from pollen masses of *X. nasalis*. The “major” pollen sources: Fagaceae, *Castanopsis* sp. (3a); Elaeagnaceae, *Elaeagnus* cf. *latifolia* (3b); Fabaceae, *Cassia* sp. (3c and 3d), *Senna siamea* (3e and 3f); Acanthaceae, *Thunbergia* (3g and 3h).

Table 3. Dominant pollens foraged by *X. nasalis*; Dominant pollen types from 14 pollen masses determined by the highest percentage pollen grain count and percentage of pollen volume (sequential order starting from the pollen mass number in the inner most cell (#1) proceeding to the nest entrance).

Nest Number/ Pollen Mass number	Family: Genus/Species	Percentage of pollen grains	Percentage of pollen volume
1/1	FAGACEAE: <i>Lithocarpus</i>	83.3	52.6
1/2	FAGACEAE: <i>Lithocarpus</i>	70.1	54.5
1/3	FAGACEAE: <i>Castanopsis</i>	55.5	15.7
	FABACEAE: <i>S. siamea</i>	22.1	41.5
1/4	ELAEAGNACEAE: <i>E. cf. latifolia</i>	56.4	87.7
1/5	ELAEAGNACEAE: <i>E. cf. latifolia</i>	76.5	65.1
2/1	ANACARDIACEAE	33.6	49.9
3/1	ELAEAGNACEAE: <i>E. cf. latifolia</i>	31.1	50
3/2	FAGACEAE: <i>Lithocarpus</i>	63.8	54.9
4/1	FABACEAE: <i>S. siamea</i>	47.0	73.8
4/2	ARACEAE	11.3	23.5
4/3	EUPHORBIACEAE: <i>Croton</i>	18.2	63.6
5/1	LYTHRACEAE: <i>Trapa</i>	49.6	74.8
5/2	FAGACEAE: <i>Lithocarpus</i>	45.5	37.3
	THEACEAE: <i>Schima</i>	14.1	55.7
6/1	FAGACEAE: <i>Lithocarpus</i>	45.2	12.2
	EUPHORBIACEAE: <i>Croton</i>	8.4	42.1

Table 2). Minor pollen sources that can be identified are of the families Araceae (genus unknown; 0.81%), Caprifoliaceae (*Sambucus*; 0.17%), Cyperaceae (genus unknown; 0.03%), Juglandaceae (*Engelhardtia*; 0.22%), and Rhamnaceae (*Ziziphus*; 0.12%); whereas nine minor pollen types could not be identified.

The dominant pollen types based on both the highest percentage pollen type amount and total percentage of pollen volumes for each of the 14 pollen masses is displayed in Table 3. Eight different families of plants were found to be the dominant contributor to the 14 pollen masses based on the highest total percentage of pollen volumes – Anacardiaceae (genus unknown), Araceae (genus unknown), Elaeagnaceae (*Elaeagnus cf. latifolia*), Euphorbiaceae (*Croton*), Fabaceae (*Senna siamea*), Fagaceae (*Lithocarpus*), Lythraceae (*Trapa*), and Theaceae (*Schima*).

Not only does *Xylocopa nasalis* display polylecty as indicated by results of the pollen analyses in its foraging behavior, but each female also exhibited a broad host plant range when foraging for pollen. Table 4 shows a foraging female that utilized 13 different pollen types to construct 5 pollen masses in a single nest, with the dominant pollen source for each pollen mass changing over time, e.g., Cells 1 and 2 are dominated by *Lithocarpus*, whereas Cell 4 and 5 are dominated by *Elaeagnus cf. latifolia*. *Castanopsis* pollens are found throughout all five pollen masses.

We also observed some notable nest-entrance behaviors by the bees. Competition for nests seemed to be very high at the nest site despite the abundance of available bam-

Table 4. Pollen composition from a single nest of *X. nasalis*; Pollen composition from a single nest (nest #1; Table 3) of *X. nasalis*. P represents a percentage of the given pollen grains in a pollen mass, whereas V represents a percentage of the pollen volume. Cell numbers are arranged from as in Table 3.

Family:	Cell 1		Cell 2		Cell 3		Cell 4		Cell 5	
Genus/Species	P	V	P	V	P	V	P	V	P	V
FAGACEAE										
<i>Castanopsis</i>	5.3	2.7	17.8	11.1	55.5	15.7	29.3	11.5	12.9	2.9
<i>Lithocarpus</i>	83.3	52.6	70.1	54.5	-	-	-	-	-	-
FABACEAE										
<i>Cassia</i>	9.8	42.6	-	-	14.2	34.4	-	-	-	-
<i>S. siamea</i>	-	-	-	-	22.1	41.5	-	-	-	-
ELAEAGNACEAE										
<i>E. cf. latifolia</i>	-	-	-	-	-	-	56.4	87.7	76.5	65.1
CAPRIFOLIACEAE										
<i>Sambucus</i>	1.6	2.1	-	-	2.5	1.7	-	-	-	-
THEACEAE										
<i>Schima</i>	-	-	5.3	19.5	-	-	-	-	-	-
ACANTHACEAE										
<i>Thunbergia</i>	-	-	-	-	-	-	-	-	5.4	31.2
TRAPACEAE										
<i>Trapa</i>	-	-	2.1	14	2	6.1	-	-	-	-
UNKNOWNNS										
Triangular and tripolate	-	-	2.6	0.5	1.0	<0.1	-	-	-	-
Triangular and inaperture	-	-	2.1	0.4	-	-	-	-	-	-
Three furrows triangular and tricoplate	-	-	-	-	1.2	<0.1	7.6	0.5	-	-
Three furrows and tricoplate	-	-	-	-	2.5	0.5	6.7	0.3	-	-
Irregular and inaperture	-	-	-	-	-	-	-	-	5.2	0.8

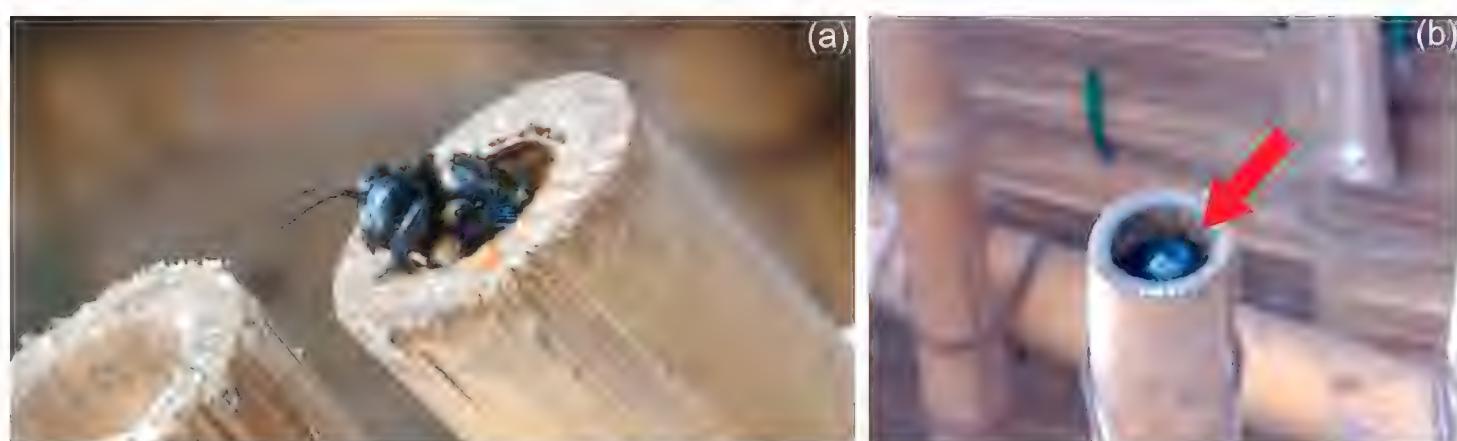


Figure 4. Nest defending postures of *X. nasalis*; Defending posture tactics performed by females *X. nasalis* to repel other conspecifics in the aggregated nesting site. The bee blocking the entrance via protruding her head out from the nest entrance (4a). Guarding the entrance by using the dorsal side of her metasoma to block the invaders (4b).

boo culms. Two defending posture tactics were observed. The most common defense posture is that of a female blocking the entrance with her head (Figure 4a), although sometimes we observed a female bees using her metasoma to block the nest entrance (Figure 4b).

Discussion

Boontop et al. (2008) provided a brief nest architectural description of 20 *Xylocopa nasalis* communal nests collected from Nakhon Pathom province ~100 km southeast of our collecting site, though they only reported the nest total lengths (described as “Internode length”), size of the nest entrances, diameter of the bamboo nests, and the sex ratio. Here, we also reported additional detailed nest characteristics that were undescribed previously. The total nest length averaged at 38.35 cm, whereas Boontop et al. (2008) found theirs to be 32.63 cm. The branch diameters of the nests are also similar between our work and that of the previous authors, 17.94 and 15.60 mm, respectively; however, the sex ratio between female and male bees from this observation is about twice to what was earlier described (8♀: 1♂ vs. 4♀: 1♂). The difference in the number of female to male bees can be explained by the collecting date, which may correspond to a later period of colony development, where most of the sister bees have emerged and stay together inside the nest, whereas male bees may have departed right after emerging from their cocoons or there is a sex ratio bias in egg-laying by the mothers. Observations on such activity are needed to test these hypotheses about the skew sex ratio in the nest. The three unconsumed pollen masses have an average fresh weight of 1.37 ± 0.13 g compared to 1.16 and 1.09 g in *X. (Ctenoxylocopa) sulcatipes* Maa, 1970 and *X. (Koptortosoma) pubescens* Spinola, 1838, species found in the desert area of the Middle East (Gerling et al. 1989).

Maeta et al. (1985) reported finding of a nesting aggregation of another *Biluna* species, *Xylocopa tranquebarorum tranquebarorum*, in Szechungchi near Henchun, Taiwan. This *Biluna* species also nested in bamboo culms though the nest entrances were excavated exclusively from the underside (the authors found only five nests in successive internodes of a single culm. In contrast to the previous finding, we found the nest entrance of *X. nasalis* to be mostly at the end of the bamboo culms, but we also observed that a couple of the nest entrances were on the underside of the culms excavated by the bees as well. This observation suggests that both *X. nasalis* and *X. tranquebarorum tranquebarorum* can excavate nest entrance from the underside of the bamboo culms, which might be a behavior shared by members of the subgenus *Biluna*. This tedious nest entrance excavation of a smooth and hard surface such as the sides of bamboo culm may be explained by the compensation that the bees will receive after the initial perforation of the culm with the omission of the need for later heavily burrowing (Iwata 1938). However, if the ends of the bamboo culms are open and

exposed, the bees may choose not to allocate their energy in the excavation of the undersides of the culms as seen in this study.

Xylocopa nasalis is polylectic with a diverse group of pollens collected. This is consistent with the described foraging behaviors of other carpenter bee species (Hurd and Moure 1963, Gerling et al. 1989, Burgett et al. 2005). Interestingly, the family Fagaceae, particularly of the genera *Lithocarpus* and *Castanopsis*, constitutes abundant pollen sources for *X. nasalis*. Both pollen types can be accounted for 26.43% and 13.77% , respectively, in terms of total grains count; however, their pollen sizes are twice or thrice smaller than other pollen types found in this study (Table 2), thus the total percentage pollen volumes contribute to the bee diets are rendered to only 7.65% and 3.22%. *Lithocarpus* and *Castanopsis* are evergreen genera of large shrubs and trees that can reach more than 20 m in height. Records show that there are 56 species of *Lithocarpus* and 33 species of *Castanopsis* indigenous to Thailand (Phengklai 2006), though there is no available information pertaining to a reliable identification of the pollen species of both plant genera in the area of study. Many species of the lowland *Lithocarpus* and *Castanopsis* flowers bloom abundantly during the beginning of the dry season to the end of monsoon period (March–October), which corresponds to the time of our collecting. It is known that species of both *Lithocarpus* and *Castanopsis* are pollinated via insects (Nixon 1989, Manos and Steele 1997, Manos et al. 2001), therefore it is evident that we should consider *X. nasalis* as one of the important pollinators for genera of large endemic trees that constitute the deciduous and evergreen landscape in the area of central Thailand. In addition, Burgett et al. (2005) reported the importance of *Lithocarpus* and *Castanopsis* pollens as two of the top food sources of the night-flying carpenter bee *X. (Nyctomellita) tranqueberica* (Fabricius, 1804) as well. They suggested that these pollen types serve as the primary pollen sources for *X. tranqueberica* found in northern Thailand, second to the introduced plant species of *Casuarina* Linnaeus, 1753, which is heavily planted throughout Thailand for reforestation (Burgett et al. 2005).

Another group of large trees that also benefit from *Xylocopa nasalis* visitation is *Senna siamea* (Fabaceae), and other related but unidentified species in the genus *Cassia*. *Senna siamea* is an indigenous evergreen tree found throughout Thailand and other neighboring countries in South and Southeast Asia; locals use its leaves mainly for consumption; it is seldom used as fodder for animals and intercropping. The flowering period of this species is documented to be during March to September or otherwise year round, if the hot and humid weather permitted (Hanum and van der Maesen 1997, Sosef et al. 1998). Both *S. siamea* and *Cassia* have poricidal anthers, which require a sonication or “buzz-pollination” from floral visitors to extract pollen from their anthers and thus eventually affect pollination (Buchmann and Hurley 1978, Buchmann 1983). Visitations by carpenter bees, which are known for their abilities to vibrate their thoraces at the pores of the flowers’ anthers to release the pollens (King et al. 1996, King and Buchmann 2003), are crucial for the reproductive successes in these plant genera. *Xylocopa nasalis* may as well be an important pollinator of this group of large trees in this area.

From the analyses of the pollen volume, we found that *Croton* (Euphorbiaceae) contributes the highest volume (14.95%). It is important to note that though this genus was found for only 2.80% of the total pollen count (Table 2), the relatively large size of *Croton* makes it become one of the most important food sources for *Xylocopa nasalis*. In Thailand, there are about 30 species of *Croton* (Chayamarit and Welzen 2005). The genus has a reputation of containing biomedical-active compounds such as alkaloids and terpenoids (Rizk, 1987) that have potential values to the pharmaceutical industries.

One genus of an annual floating-leaved aquatic plant is also frequently visited by *Xylocopa nasalis*. The pollens of water chestnut of the genus *Trapa* (Lythraceae) contribute 13.36% of the total pollen volume in the bee diets. Smitinand (2001) listed only three *Trapa* species in Thailand: *T. bicornis* Osbeck, 1771, *T. incisa* Siebold & Zuccarini, 1845, and *T. natans* Linnaeus, 1753. However, the taxonomy of *Trapa* is still in flux (Kadano 1987; Cook 1996; Takano and Kadano 2005), and a thorough survey of this common aquatic plant in Thailand is needed, since the fruits of *T. bicornis* are one of the important food crops in Thailand. Identification of *Trapa* pollens to specific level can provide important information regarding which species *X. nasalis* visit and pollinate.

Lastly, the main shrub species that *X. nasalis* visits for pollen is *Elaeagnus* cf. *latisolia* (Elaeagnaceae), a prominent shrub that has a native range in northern Thailand, although it can be found throughout the country due to its high adaptability to various soil conditions and habitats (Smitinand 2001). Other minor pollen-providing plants that can be identified in this work such as *Sambucus* and *Ziziphus*, which are possibly introduced into the area as ornamental plants, and which contribute less than 1% of the pollen volume in a given pollen mass.

Our observations of nest-defending by resident females are consistent with the nest defending postures reported in *Xylocopa sulcatipes* and *X. pubescens* in Israel (Gerling et al. 1989) and in *X. (Ctenoxylocopa) fenestrata* (Fabricius, 1798) in India (Kapil and Dhaliwal 1968a), though whether the guarding females are the progeny of the founding female still needs to be investigated in *X. nasalis*.

Conclusion

In summary, our observations and dissections of *Xylocopa nasalis* nests agree with known reports of other *Xylocopa* species (Hurd 1958, Hurd and Moure 1960, 1961, 1963, Kapil and Dhaliwal 1968a, 1968b, Michener 1974, Mordechai et al. 1978, Gerling et al. 1989, Boontop et al. 2008). The *X. nasalis* nest is strictly unbranched. The provisioned cells are separated via partitions made from bamboo particles excavated by the founding female. The nesting architectural details provided within this work should prove to be of beneficial to beekeepers and researchers who are interested in trapping and studying *X. nasalis*. For further genetic and social behavioral studies, we found that in a given nest, sister bees can tolerate and live inside the same nest with up to 7 individuals along with their mother. Kinship analyses using molecular mark-

ers such as microsatellite DNA will reveal interesting details pertaining to the social structure in a single nest and the population structure of the bees living communally in the same vicinity in Ratch Buri province, Thailand (W. Hongjamrassilp and N. Warrit (unpublished data)). As for the pollens foraged by *X. nasalis*, the broad host plants range can be highly beneficial for many crop pollinations, particularly for plants that require the “buzz” pollination method by their pollinators (Keasar 2010). Flower constancy and other related pollination studies are required for further justification of using *X. nasalis* as future potential pollinator for agricultural and forest plants in Southeast Asia.

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